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ON THE RELATION OF THE BLOOD SALTS TO CARDIAC CONTRACTION

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THE importance of the salts of the blood in maintaining the beat of the heart is established beyond question. The precise relation of individual salts in the series of reactions making up a heart-beat is still the subject of study. The problem of the action of salts cannot be attacked directly; too many factors are involved. Conclusions must be drawn, therefore, through comparison of data obtained from numerous more or less indirect experiments. The difficulties of interpretation under these conditions are necessarily very great, and workers have been led to form very diverse opinions from quite similar data.

Consider, for example, the question of the "inner stimulus," the actual excitant to the cardiac contraction. At least three distinct views as to its nature are at present before physiologists. Lingle,[1] correlating heart tissue with skeletal muscle tissue, adopts Loeb's view that sodium ions constitute the excitant. Langendorff [2] looks upon the metabolic products of the heart's own activity as the means

[1] Lingle: this Journal, 1900, iv, p. 265.

[2] Langendorff: Archiv für Anatomie und Physiologie, physiologische Abtheilung (Suppl. Band), 1884, p. 1.

of stimulation. Howell [3] suggests that the origination of the beat is not dependent on any specific stimulus, but results from the spontaneous break down of the highly unstable substance whose decomposition yields the energy manifested in the contraction.

With regard to the rôle of individual salts there is a like difference of opinion. Thus, in respect to calcium, Lingle [4] believes that this salt exerts its beneficial effect by antagonizing the poisonous action of sodium. S. R. Benedict [5] attributes the improved beat which follows its use to the rise in tone which it brings about. Howell [6] pictures the rôle of calcium in connection with the conversion of energy-liberating substance from a stable into an unstable, easily dissociable compound. From a study of the relation of the tissue to its oxygen supply I was led [7] formerly to believe that calcium might have something to do with the oxidative activities of the heart substance.

Purpose of this Study. — My intention, in presenting the views herein contained, is not to advance any new theory of salt action, but to suggest a means whereby the divergent theories already current can be harmonized in a fashion that will offer satisfactory explanations of the various salt-phenomena thus far described, so, perhaps, simplifying the situation.

In studies of the action of salts on the heart the chief consideration hitherto has been the presence or absence of spontaneous rhythmicity. Solutions have been classified according as they favor or interfere with contraction. Series of beats have been examined to see whether the media used cause exhaustion or recovery. A somewhat different, and perhaps instructive point of view can be gained if attention is directed to the character of the individual contractions. The operation of the "all or none" law of cardiac activity makes the height of any contraction the index of the amount of energy-liberating material available for performing it. By observing the effect of various solutions on the height of contraction, therefore, conclusions

[3] HOWELL: The Harvey Lecture. Journal of the American Medical Association, 1906, xlvi, No. 23.

[4] LINGLE: *Loc. cit.*

[5] BENEDICT: this Journal, 1905, xiii, p. 199.

[6] HOWELL: *Loc. cit.*

[7] MARTIN: this Journal, 1906, xvi, p. 214.

may be drawn as to the influence of the solutions on the elaboration of dissociable energy-yielding material within the tissue.[8]

Schultz[9] has described another means of gaining information as to the effect of salts on heart tissue, namely by determining their influence on the time required after contraction for the excitability to electric stimuli to return to normal.

By the use of this criterion of Schultz and the one I suggest above, in connection with the older criteria, which are concerned primarily with the efficiency of media in causing beats, we should be able to differentiate, if there be any difference, between agencies concerned with the actual production of contractions and those having to do rather with the preliminary process of preparing dissociable material.

Is there a Specific Inner Stimulus? — That the immediate process of contraction depends upon a definite inner stimulus, in mammalian hearts, at least, seems to be pretty clearly established by some recent observations of Cushny[10]. This author stimulated a spontaneously beating ventricle at a rate faster than its own, forcing it into an abnormally rapid rhythm. At the cessation of the artificial stimulation the ventricle showed a period of stand-still before resuming spontaneous activity. The significant observation of Cushny was that during this period of stand-still the heart was as sensitive as at other times to artificial stimulation. The stand-still was not due, therefore, to a loss of irritability, but to a failure of the inner stimulus; and the experiment demonstrates the existence of an inner stimulus quite independent of any particular condition of irritability. Hering[11] has also argued in favor of a specific inner stimulus. He cites various experiments in support of his view, but considers two observations particularly conclusive. The first of these is the reversal of rhythm sometimes seen in perfused mammalian hearts, whereby the auricles,

[8] If the height of contraction is to be interpreted in this manner care must be used that the tissue under examination is contracting as a whole, and not in part only. The tendency of heart tissue under experimental conditions to show partial contractions is pointed out by Schultz. (This Journal, 1908, xxii, p. 134.) I have observed the same repeatedly. There is, however, little difficulty in distinguishing partial from complete contractions if one is on the lookout for them.

[9] SCHULTZ: this Journal, 1908, xxii, p. 133.

[10] CUSHNY: Heart, 1912, iii, p. 257.

[11] HERING: Archiv für die gesammte Physiologie, 1911, cxliii, p. 370, and 1912, cxlviii, p. 608.

instead of setting the pace, beat in response to stimuli from the spontaneously-beating ventricles. This, according to Hering, is an example of auricular irritability maintained in the absence of spontaneous activity. Hering's second significant observation is that in the dying heart the various parts become inactive, not together, but one after the other. This is interpreted as signifying that in this case loss of irritability precedes failure of the inner stimulus.

Although the existence of a definite inner stimulus seems, from Cushny's observations, to be satisfactorily established, the experiments of Hering do not appear particularly conclusive in support of it. The failure of the auricles in the perfused heart to beat spontaneously can be as reasonably explained by assuming a partial loss of irritability, sufficient to prevent spontaneous activity, but not to abolish response to stimuli from the beating ventricle, as by assuming an unimpaired irritability made ineffective through the disappearance of the inner stimulus. In the observation on the dying heart the successive failure of region after region shows, it is true, a progressive loss of irritability, but to stimuli proceeding from the regions still active; there is nothing in the experiment to prove that there is an inner stimulus exerting its influence ineffectively in the inactive regions. Hering [12] has pointed out a possible fallacy in such experiments as that of Cushny, in that in them the criterion of cardiac irritability is responsiveness to artificial stimulation, a criterion which may not be valid when applied to the irritability of the heart for its normal stimulus. To settle the question finally, therefore, either responsiveness to artificial stimulation must be shown to be a valid criterion of cardiac irritability, or some other undoubted index of irritability must be established.

Various considerations incline me to the view that cardiac irritability depends upon the amount of available energy-liberating material, per unit of substance, present in the tissue. It is universally assumed that in the rhythmically beating heart at the end of systole the available source of energy is exhausted, and that during the succeeding diastole there is an accumulation of the special material required for yielding the energy of the next systole. That during this accumulation there is also a steady increase of irritability until the beginning of the next systole is also generally assumed.

[12] HERING: *Loc. cit.*, 1911, cxliii, p. 376.

If we grant that in the rhythmically active heart the diastolic accumulation of energy-yielding material is accompanied by increasing irritability, must we not admit the probability, at least, that the presence of abundant available material in the quiescent heart signifies high irritability there as well?

The index to the amount of energy-liberating material available for any contraction is, as stated earlier, the height of the contraction. If the increase in irritability goes hand in hand with the accumulation of this material, we can judge the degree of irritability by the same criterion. As indicating that this suggested relationship between contraction height and irritability actually holds, certain experimental results are interesting, even though they are based on the

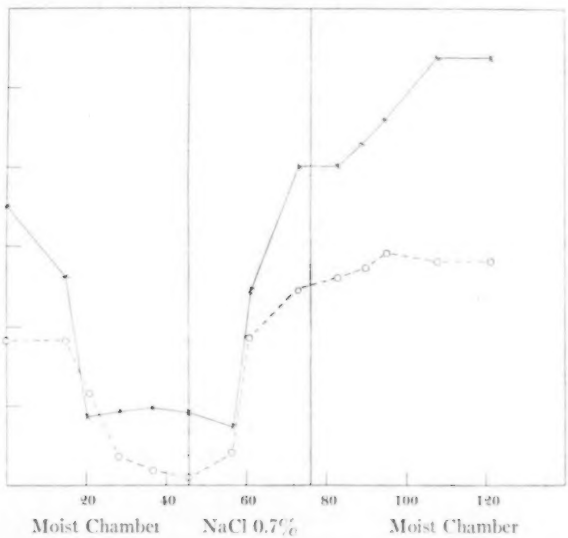

FIGURE 1. Curves showing that height of contraction and excitability to induction shocks vary in parallel fashion in freshly excised ventricle strips. The continuous line is the curve of irritability; the broken line is the curve of contraction height.

results of artificial stimulation, and cannot, on that account, according to Hering, be accepted as wholly convincing.

Schultz reports [13] that freshly-cut heart strips immersed in Ringer's solution give, for some time after immersion, progressively higher

[13] SCHULTZ: *Loc. cit.*, p. 143.

contractions when stimulated, and during this same period show, according to his criterion, mentioned above (p. 167), progressively increasing irritability. By a method recently developed [14] I have made accurate determinations of the threshold of response to induction shocks of freshly-cut heart strips, and find that there is a remarkable parallelism between the curve of irritability and of contraction height. This parallelism is shown graphically in Fig. 1, which is the record of the course of the irritability and the height of contraction of a strip during the first two hours after excision.

Schultz reports, and I confirm his statement, that in heart tissue several hours after removal from the body, the agreement between excitability to electric stimuli and height of contraction seems not to hold so strictly. Even though there is thus some opposing evidence, the preponderance favors the idea that height of contraction is on the whole a fairly good index to the excitability of the tissue, and we are

FIGURE 2. Tracing showing that a heart strip which is not spontaneously active may give under artificial stimulation, contractions which are much higher than previous spontaneous contractions of the same strip.

justified, therefore, in basing tentative judgments as to irritability on observations of contraction height.

Let us now apply this criterion of irritability to the problem of the inner stimulus: Can we show that strips which are spontaneously active with a certain degree of irritability may lose their spontaneity and yet develop even greater irritability? Fig. 2 is a tracing obtained from an apex strip of ventricle from Chrysemys marginata. The tracing was taken Nov. 21 and 22, 1911. The fresh strip was immersed in 0.7 per cent sodium chloride solution till beats began. The latent period was one hour and ten minutes. Five minutes after the onset of spontaneous rhythmicity the sodium chloride solution was replaced (2.15) by 50 c.c. Ringer's solution (NaCl 0.7%,

[14] MARTIN: this Journal, 1908, xxii, p. 116. Also the Measurement of Induction Shocks, New York, 1912.

$CaCl_2$ 0.026%, KCl 0.04%), to which 2 c.c. 0.9 per cent potassium chloride had been added. Spontaneous activity immediately ceased. At intervals, as indicated on the tracing (2.25, 2.35, 2.50, 3.05, 3.30 P.M and 10 A.M), series of three minimal induction shocks were sent through the strip. The liquid was drawn off just long enough in each case for the stimulations to be sent in. The record shows a progressive increase in the height of contraction from series to series, continuing for twenty hours after the first activity of the tissue. The first contractions under artificial stimulation were higher than the highest spontaneous contractions, and the last contractions under artificial stimulation were twice as high as the spontaneous contractions. This experiment, which I have performed repeatedly with similar results, proves that a procedure which prevents spontaneous activity completely may have no influence on the development of irritability, and to that extent justifies Cushny's use of artificial stimuli in judging the irritability of the quiescent mammalian heart in his experiment, cited above. Another experiment, described below, seems to me to give positive indication that spontaneous beats, when they do occur, are the result of the operation of a definite "inner stimulus." In this experiment I was determining the threshold of irritability of ventricle strips to single induction shocks, measuring the stimuli by the method I have recently described.[15] A strip had been immersed in 0.7 per cent sodium chloride solution till spontaneous beats began, and was then removed to moist air. Spontaneous activity ceased in about five minutes, without any indication of the onset of "sodium chloride exhaustion." After fifteen hours in the moist air the threshold stimulus for the strip was 720 Z units.[16] The contractions following this stimulation were very vigorous Seven minutes after determining the threshold the strip was immersed in 0.7 per cent sodium cholride. It contracted spontaneously in less than one minute; the sodium chloride was then withdrawn. One more spontaneous beat occurred, and then the strip remained quiet. Three minutes later the threshold was 660 Z units. Five minutes later still the threshold had fallen to 528. Five minutes after this last stimulus the strip was again immersed in 0.7 per cent sodium chloride solution. Strong spontaneous contractions began within

[15] MARTIN: *Loc. cit.*

[16] MARTIN: this Journal, 1910, xxvii, p. 228.

ten seconds. These continued for fifteen minutes, although the sodium chloride solution was withdrawn after one minute. Eight minutes after the strip had ceased spontaneous activity the threshold was still 528. It began to decline soon after, however, and within a half hour threshold values as low as 317 were obtained, without any reappearance of spontaneous beats. During the hour in which the observations just cited were made, a ventricular strip had a declining threshold of irritability, virtually throughout the period. It showed spontaneous activity twice; each time immediately after the application of a sodium chloride solution; and each time upon a different plane of irritability. To one watching a strip behaving as this one did, the impression is overwhelmingly of a definite stimulus thrown into operation with each immersion in the sodium chloride solution.

The Characteristic Features of Ventricular Activity in Pure Sodium Chloride Solution. — Careful study of a large number of experiments in which ventricle strips have been placed in pure sodium chloride solutions after various sorts of preliminary treatment, has impressed me with certain features of sodium chloride action which seem to be general, and which I would summarize as follows: (1) *For ventricle tissue to be active in sodium chloride solution, it must come from a medium favorable to the production of dissociable substance.* (2) *The onset of activity is prompter the greater the irritability in the preceding medium.* (3) *The first spontaneous contractions in sodium chloride equal in height the last contractions in the preceding medium.*

Leaving out of account, for the moment, the effects of sodium chloride on freshly-excised strips, the conditions favorable for the manifestation of the sodium chloride effects outlined above are: long continued immersion in Ringer's solution; prolonged suspension in moist air following immersion in sodium chloride solution; sometimes treatment with sugar solution after sodium chloride exhaustion.[17]

The usual termination of spontaneous activity in these preparatory media is through a period of lessening frequency of beat, without much decline in height of contraction, indicating a diminished spontaneity, but not a decrease in the production of dissociable substance. The effect of immersion in pure sodium chloride solution is in every one of these cases prompt resumption of rhythmic activity — after

[17] For detailed description of the behavior of strips in the media named, *see* MARTIN: this Journal, 1904, xi, p. 123 *et. seq.*

Ringer's solution activity is usually resumed with striking suddenness, and in every case the first beats are the highest of the series and are about the same height as the last beats in the preceding medium.

Frequently strips in moist air, particularly when placed therein after brief treatment with sodium chloride [18] give a rather long series of beats with continuously declining vigor of contraction. Immersion in sodium chloride solution at the end of such a period sometimes brings about a series in which there is gradual increase of vigor for some time, as long as fifteen minutes in my experiments. This result is significant in that it forms the only exception I have seen to the general observation that the first beats in sodium chloride solution are maximal or nearly so.

One of the very familiar phenomena attending the use of sodium chloride on heart strips is the rather long latent period which precedes activity, when the strips are immersed in the solution immediately after removal from the animal. The various explanations of this latent period that have been offered need not be reviewed here. I wish, however, to report certain facts about the condition of heart tissue immediately after separation from the animal, including the latent period in sodium chloride. Schultz [19] reports that heart strips cut while in Ringer's solution and suspended in moist air are inexcitable to induction shocks for a half hour or longer. I have studied the excitability of similar strips, the only difference between my procedure and that of Schultz being that I invariably omitted any rinsing solution; the strips were always cut directly from the heart, through which blood was being pumped. Such strips, placed for a moment on a glass plate, are very irritable to mechanical stimulation and in responding to the stimuli unavoidable in handling them, pump themselves quite free of blood. My observations differ from those reported by Schultz in that they show that the freshly isolated strips, when first placed in moist air, are fairly excitable to induction shocks. I have records of threshold stimuli ranging from 290 to 850 Z units. In my experience the irritability declines steadily. In an experiment in which the initial threshold was 290 it had increased in twenty minutes to 1100. In an experiment with a high initial threshold, 850 Z units,

[18] *See* MARTIN: *Loc. cit.*, p. 124.

[19] SCHULTZ: *Loc. cit.*, p. 134.

no contractions were given after twenty minutes by stimuli of 8000 units.

I have demonstrated also, by means of an experiment described in my first paper on this subject,[20] the steady decline in irritability in moist air of freshly isolated apex strips. In this experiment the entire heart is removed from the body and suspended in moist air and the apex is partially severed from the base. Records of the contractions of the apex are taken. This procedure gives an apex strip, virtually isolated so far as salt relationships are concerned, but subject to rhythmic stimulation from the active venous portion of the heart. Such strips invariably show a steady decline in height of contraction, corresponding, we must believe, with a steady diminution in production of dissociable material, and with steady decline in irritability. The onset of complete "exhaustion" usually requires about two hours.

However we may interpret these observations they demonstrate the fact that ventricular tissue upon separation from its normal environment loses its excitability more or less rapidly. When a strip is immersed in sodium chloride solution it has first to overcome this tendency to declining irritability before it becomes able to execute spontaneous contractions.

Schultz (*loc. cit.*) states that the return of excitability of excised strips is promoted by immersing them in a saline bath. I have made observations of the threshold stimulus, at intervals during the latent period, of fresh strips in 0.7 per cent sodium chloride, the solution being withdrawn only long enough in each case to allow stimulation. I find that the threshold a few minutes after immersion is usually several times higher than at the moment of separation from the body. Thus in one experiment, the threshold of the freshly isolated strip was 400 Z units. Five minutes after immersion in 0.7 per cent sodium chloride the threshold was 1224. In seven minutes more the threshold had fallen to 576; eight minutes later it was 343; and three minutes after this reading, and just before spontaneous contractions began, the threshold was 300. Similar variations, although with a more rapid secondary increase in irritability, I have observed in fresh strips immersed in Ringer's solution. Such results show that removal from the body to any medium whatever involves in the course of the readjustment of the tissue to its environment a lowering

[20] MARTIN: this Journal, 1904, xi, p. 105.

of excitability, which must be overcome before activity is resumed. I believe the necessity for this readjustment explains, at least, in part, the long latent period of fresh strips in sodium chloride solution, as well as the various means of shortening the period that have been described. Further discussion of this point is reserved for a later portion of the paper.

The Characteristic Features of Ventricular Activity under Treatment with Calcium-Containing Solutions. — Apex strips respond to treatment with solutions containing calcium in a manner perfectly characteristic, and often very striking. The effect of such solutions, when positive, is always to bring about a marked increase in the *height* of *contraction*, which is prompt in showing itself. I have seen the effect follow the addition of calcium-containing solutions to sodium chloride solutions surrounding strips in all stages of the typical sodium chloride series. Strips beating in moist air show marked increase of vigor after the application of calcium-containing solutions. Fresh strips immersed in Ringer's solution show only occasional spontaneous contractions, but such as occur are always very vigorous indeed.

That this effect of calcium extends to mammalian heart tissue is shown by Langendorff and Hueck [21] and by Gross,[22] who report that a permanent increase in the calcium content of the circulating blood causes a permanent increase in the force and amplitude of the heart beat.

Discussion

I have emphasized the characteristic effects of sodium and of calcium on ventricular tissue to bring out what I assume, tentatively, to be their respective functions with reference to cardiac activity. The effect of sodium is always such as to suggest the action of a direct stimulus, and this I assume to be its function so far as the heart is concerned. In adopting this view I am abandoning my former position [23] of support for the Langendorff theory of stimulation by metabolic products, in favor of Lingle's theory that sodium ions consti-

[21] Langendorff and Hueck: Pfluger's Archiv, 1903, xcvi, pp. 473–485.

[22] Gross, E.: *Ibid.*, 1903, xcix, pp. 264–322.

[23] Martin: this Journal, 1906, xvi, p. 201.

tute the "inner stimulus"; but only to the extent of granting to sodium ions a positive stimulating effect. I still believe that the evidence formerly adduced by me [24] indicates that metabolic products may also stimulate heart tissue. I see no reason why we must confine stimulating properties to single substances, excluding all others. Indeed an experiment reported by Benedict,[25] in which galactose solution caused beats in a freshly isolated apex strip, and the commonly observed revival after sodium chloride exhaustion by sugar solutions, can be explained more satisfactorily by granting to these substances certain stimulating powers than in any other way.

An objection which may be urged against this theory, so far as it assumes for sodium a direct stimulating function, is that in such an experiment as that cited on p. 171, in which immersion in sodium chloride is followed promptly by beats, the tissue must be saturated with sodium chloride at the time of immersion, and the application of more sodium to a tissue already saturated with it would scarcely be expected to exert a very positive influence.

To my mind the best answer to this objection is the experiment itself. The tissue, saturated with sodium chloride, is quiescent; when immersed in more sodium chloride it beats. Obviously there is some difference of condition before and after immersion. The equilibrium existing in the tissue saturated with sodium chloride is instantly upset when it is immersed in more sodium chloride.

We have no positive knowledge as to the ionic conditions obtaining in the equilibrium of the quiescent tissue. Various hypotheses to account for the equilibrium and its upset by sodium chloride might be offered. A suggestive fact is that equilibrium with quiescence cannot prevail in heart tissue *immersed* in sodium chloride solution, after the initial latent period is over, so long as dissociable material is available, and provided activity is not prevented by the presence of an inhibitory substance, such as potassium. Moreover, after prolonged immersion in sodium chloride solution, strips removed to moist air are nearly always active for many hours. In view of these facts the occasional stand-still shown by strips, after a rather brief immersion in sodium chloride solution, suggests that a true satura-

[24] MARTIN: *Loc. cit.*, pp. 203 and 205.

[25] BENEDICT: this Journal, 1908, xxii, p. 22.

tion may not have occurred under these conditions, and lessens the force of the objection I have mentioned.

The effect of calcium seems clearly to be that suggested by Howell,[26] namely to promote the production of dissociable energy-liberating material. If the height of contraction is a reliable index to the amount of dissociable substance available, as we must believe it to be, all the reported facts about the effects of calcium point directly to this conclusion, for the one striking feature of the calcium effect wherever it appears is improvement in the vigor of beat.

The dependence of the calcium effect upon the oxygen supply, which, as I have previously shown,[27] is very marked, I formerly interpreted [28] as indicating a rôle for calcium in connection with the oxidative processes in the tissue. That the facts can be interpreted as satisfactorily in terms of my present view I shall show in a later paragraph.

The theory of the action of sodium and calcium on heart tissue presented in this paper may be summarized in a brief statement. Sodium and calcium ions are not antagonistic, but act positively upon different phases of the contractile process. Calcium promotes the conversion of stable material into unstable, energy-liberation material; sodium promotes the dissociation process whereby energy is actually liberated. In other words, calcium makes material available, sodium causes this material to liberate its energy. In neither of these functions are the salts exclusive agents; the production of dissociable material may depend on other factors than calcium; the dissociation process may be affected by other substances than sodium.

The notion that sodium and calcium ions are antagonistic in their influence on vital processes has prevailed since the work of Ringer [29] and of Loeb [30] on the interaction of these and other ions. The greater part of the evidence offered in favor of such antagonism has been derived from experiments on tissues other than heart, chiefly skeletal muscle, Fundulus, and Gonionemus. Loeb has recently presented

[26] Howell: Journal of the American Medical Association, 1906, xlvi, No. 23.

[27] Martin: this Journal, 1906, xv, p. 309.

[28] Martin: *Ibid.*, p. 316.

[29] Ringer: Journal of physiology, 1886, vii, p. 302; also, xviii, 1895, p. 428.

[30] Loeb: this Journal, 1900, iii, p. 337; also, Archiv für die gesammte Physiologie, lxxx, 1900, p. 229.

his ideas on salt antagonism,[31] taking the position that the antagonism is not a true one in the sense that one salt acts in direct opposition to another, but rather that there is a cooperative action of the salts upon the tissue, of such a sort as to interfere with the manifestation of the peculiar effect of either one. The experimental basis for Loeb's view is chiefly a series of studies on the effects of salts on developing eggs of Fundulus. Osterhout[32] has demonstrated by two distinct methods that in plant cells "the antagonistic action of salts is largely or entirely due to the fact that they hinder or prevent one another from entering the protoplasm."

Joseph and Meltzer,[33] on the other hand, have described experiments on skeletal muscle, which furnish indication that in this particular tissue there may be a direct salt antagonism.

All these observations and opinions serve to show clearly that the conclusion reached depends often on the tissue studied To assume, then, an antagonistic action in heart tissue between sodium and calcium ions, on the ground of observations made on very different tissues, is surely unwarranted.

When I reviewed the literature of the causation of the heart beat, after eliminating from my mind the idea that sodium and calcium are antagonistic, I wondered that the idea could persist so strongly with such meagre evidence in its favor. The observations upon which the belief is based, with reference to heart strips, are the recovery from sodium chloride "exhaustion" which follows the use of calcium, and the better beat in sodium-calcium mixtures as compared with pure sodium chloride solution,[34] and the observation of Howell[35] that sodium tends to relaxation, while calcium tends to tonic shortening. With reference to the improvement in beat caused by calcium, I have shown above that this is apparently a direct calcium effect, and there is abundant evidence that it may be quite independent of a previous injurious influence of sodium. Thus strips which have been vigorously active for hours in moist air often show marked

[31] LOEB: The Carpenter Lecture, Science, N.S., xxxiv, 1911, p. 653.

[32] OSTERHOUT: Science, N.S., xxxiv, 1911, p. 187; and *Ibid.*, N.S., xxxv, 1912, p. 112.

[33] JOSEPH and MELTZER: this Journal, 1911, xxix, p. 1.

[34] LINGLE: *Loc. cit.*, p. 277.

[35] HOWELL: this Journal, 1901, vi, pp. 184 and 199.

increase in amplitude following the application of calcium-containing solutions, a result which can scarcely be due to an antagonizing of sodium by the calcium.

Howell's correlation of the antagonism between sodium and calcium in the causation of the beat with their antagonism in relation to tone loses its force if the salts are shown not to be antagonistic in their effects on tone. In a recent paper [36] I have analyzed the relations of salts to cardiac tonus and have advanced evidence which seems to me to cast grave doubt on the view that sodium and calcium as present in the blood are definitely antagonistic in their influence on tone.

If there is truth in the idea herein advanced that sodium and calcium ions are not antagonistic in their effects on heart tissue, but act positively on different phases of cardiac activity, the various previous observations on the behavior of heart substance when treated by these ions must be explicable in terms of this idea. I purpose, as briefly as possible, to discuss the recorded observations on this basis so as to show the applicability to them of my hypothesis.

Ventricular tissue of the turtle in its normal relation to the blood supply we know to be highly responsive to stimuli from the venous end of the heart, but, as the immediate stand-still following excision shows, not capable of spontaneous activity. We have in this situation a high degree of excitability in the absence of an effective inner stimulus.

When ventricular tissue is removed from the blood supply, but not otherwise treated, its excitability, both to artificial stimulation and to the normal stimulus from the active venous region, diminishes steadily. This decline in excitability can be explained as due to a disturbance of equilibrium whereby the ions present in the tissue enter other than their normal combinations. A similar change for the ions of shed blood I have already suggested.[37] That the disturbance in ionic relationships is a permanent one is shown by the complete failure of excised ventricle to recover excitability unless treated with certain suitable solutions.

Freshly cut ventricle strips immersed in 0.7 per cent sodium chloride solution show a latent period in which there is at first a decline

[36] MARTIN: this Journal, 1912, xxx, p. 182.

[37] MARTIN: this Journal, 1904, xi, p. 117.

in excitability to induction shocks. This is succeeded by a period of increasing excitability, and this in turn by spontaneous activity.

Methods of shortening the latent period are by treatment with calcium-containing solutions or carbon dioxide (Martin) or sodium oxalate or galactose (Benedict). On the justifiable assumption that spontaneous beats begin as soon as the intensity of the inner stimulus meets the threshold we may look for shortening of the latent period either by agencies which intensify the inner stimulus or by those that increase the excitability. According to the theory proposed by Howell and supported in this paper the shortening of the latent period by calcium is due to the action of this substance in increasing the excitability. Inasmuch as the immediate onset of beats after sodium oxalate is said by Benedict ordinarily to require the use of Ringer's solution rather than pure saline, we may interpret the effect of the oxalate as due to the precipitation of the calcium of the tissue, thereby preparing the way for immediate influence to be exerted by the calcium of the Ringer's solution. The effects of carbon dioxide and of galactose are best to be explained, I believe, by attributing to them direct stimulating properties.

The ultimate onset of spontaneous activity of strips in pure sodium chloride solution, following a period of increasing excitability, can be explained by supposing that the tendency of the ions contained in the tissue to enter combinations unfavorable to excitability is overcome by the presence of the sodium. This possibility I have previously pointed out.[38]

The declining series of beats terminating in "sodium chloride exhaustion" signifies a continuous decline in the conversion of energy-liberating material from inactive into active form. This decline is probably due, as I have suggested elsewhere,[39] primarily to insufficient oxidation, whereby unoxidized waste products accumulate to an extent which interferes with the production of energy-liberating material. The evidence for this view is the excellent recovery which follows treatment with abundant oxygen. Since calcium salts also induce recovery from this exhaustion we must believe that under the stimulation of calcium the production of dissociable material goes on in spite of the clogging effect of the unoxidized waste products,

[38] MARTIN: this Journal, 1906, xvi, p. 203.

[39] MARTIN: *Ibid.*, 1906, xv, p. 319.

although, as I have shown,[40] the calcium effect cannot manifest itself in the complete absence of oxygen.

Sodium carbonate and sugar are other substances that bring about recovery from sodium chloride exhaustion. That induced by sodium carbonate is probably akin to the improvement which follows treatment with oxygen, at least in that it operates by getting rid of waste products, although in this case the method is neutralization and not oxidation. The beneficial effects of sugar solution may be either through influence on the conversion of material into available form, or, as seems to me equally likely, through a combination of this effect with a powerful reinforcement of the inner stimulus.

The Rôle of Potassium. — That potassium is inhibitory to cardiac activity has long been definitely established. The precise mechanism of its action is, however, unknown. I am inclined to believe that potassium in moderate concentrations acts in opposition to the inner stimulus rather than in antagonism to the elaboration of energy-liberating material, but in higher concentrations opposes both processes. Some observations that bear out this view are reported by Howell [41] in connection with studies of the relation of the potassium content of the circulating medium to vagus inhibition. Howell states that if the concentration of potassium chloride in the medium is gradually increased a point is reached (0.1 per cent) above which there is a definite effect upon the force of the beat, as well as upon its rate. In this diminution of force we have definite evidence of an effect of potassium on the production of dissociable material in the ventricle. The effects of potassium on rate are irrelevant in this connection, since the ventricular rate is established by the venous part of the heart.

That concentrations of potassium insufficient to affect unfavorably the production of energy-liberating material may, by opposing the inner stimulus, prevent the manifestation of spontaneous activity in the ventricle is shown by the experiment of which a tracing is given in Fig. 2, p. 170. In this experiment spontaneous rhythmicity developed as the result of immersion in 0.7 per cent sodium chloride solution, and after becoming well established was abolished by transferring the tissue to Ringer's solution containing a small excess of

[40] MARTIN: *Loc. cit.*, p. 316.

[41] HOWELL: this Journal, 1906, xv, p. 283.

potassium chloride. That the suspension of activity was not due to interference with the production of dissociable material was shown when artificial stimuli were applied. The responses to these stimuli were progressively greater and greater, a result which can be explained only by supposing that the presence of potassium in small concentration is no bar to the conversion of substance into energy-liberating form, although it does suffice to overcome the action of the inner stimulus.

According to the view of the action of sodium and calcium set forth above, we may look upon potassium as antagonistic to both sodium and calcium, but to sodium more markedly than to calcium, a much higher concentration of potassium being required to counteract the influence of calcium on the process of preparing energy-liberating material than to counteract the directly stimulating influence of sodium.

Summary

1. Various means of studying the influence of salts on the heart-beat are considered. The conclusion is drawn that by virtue of the "all or none" law the height of contraction is an index to the amount of available energy-liberating material present at the beginning of the contraction, and as such offers a fruitful means of attacking the problem of salt action.

2. Evidence is presented indicating that to a considerable degree the irritability of heart tissue depends on the amount of dissociable substance per unit of mass present within it, so that the height of contraction may serve to some extent as a criterion of irritability.

3. An experiment is described which may be interpreted as confirming Cushny's demonstration of the existence of a specific inner stimulus.

4. The characteristic features of ventricular activity in pure sodium chloride solutions are stated as follows: (1) for ventricle tissue to be active in sodium chloride solution the tissue must come from a medium favorable to the production of dissociable substance; (2) the onset of activity is prompter the greater the irritability in the preceding medium; (3) the first spontaneous contractions in sodium chloride equal in height the last in the preceding medium.

5. Freshly excised ventricle tissue is shown to undergo a steady decline in irritability which can be overcome only by treatment with suitable solutions. The decline occurs during the first few minutes of immersion in sodium chloride solution or Ringer's solution, but is presently succeeded in these solutions by steadily increasing irritability. The initial decline is interpreted as an inevitable effect of cutting the tissue off from its usual environment. Its occurrence explains the latent period in sodium chloride.

6. The characteristic effects of calcium-containing solutions on heart tissue are shown to be always in the direction of increased vigor of beat.

7. To interpret the characteristic effects of sodium and calcium the notion that they are antagonistic in their action on heart tissue is rejected. To each is assigned a positive function on a definite phase of the heart's activity. For calcium is assumed the function proposed for it by Howell of acting to promote the conversion of stable into unstable energy-yielding material; and for sodium the function proposed by Lingle of serving as the immediate stimulus to bring about the actual dissociation, and so to initiate the beat. To account for various observations the further assumption is made that neither sodium nor calcium is an exclusive agent; the preparation of dissociable substance is hampered to a great degree by accumulating waste products, and is therefore aided by abundant supplies of oxygen or by sodium carbonate; carbon dioxide in moderate concentration, and perhaps sugar, act to stimulate heart tissue directly, much as sodium does.

THE SUGAR CONSUMPTION IN NORMAL AND DIABETIC (DEPANCREATED) DOGS AFTER EVISCERATION [1]

By J. J. R. MACLEOD and R. G. PEARCE

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IN connection with the physiology of the carbohydrates no problem more urgently requires solution at the present day than that of the relationship which the pancreas holds to the utilization of sugar in the animal body. The hyperglycaemia which supervenes with such remarkable rapidity after complete pancreatectomy and the subsequent inability of the animal to metabolize any form of carbohydrates indicate that in the normal animal this relationship must be of the very greatest importance. When we attempt to explain the manner in which the pancreas exercises this function, however, great difficulties present themselves. We are, for example, not yet certain whether the pancreatic function is a local one or one effected in other parts of the body by an internal secretion (hormone) which the gland discharges into the blood.

By a local function is meant one by which some toxic substance, present in the blood and produced in the course of body metabolism, is acted on by the pancreatic cells in such a way as to neutralize it. When this substance is not thus acted on by the pancreas its accumulation in the blood paralyzes the power of the tissues to utilize sugar. Although there is no known fact which absolutely disproves this view there is likewise no direct evidence which would encourage us to entertain it, so that the tendency at present is to consider the pancreatic influence as being exercised by means of an internal secretion. Even if we accept this view, however, there is no agreement as to the *modus operandi* of the hormone. Some believe that the hormone is necessary for the utilization of dextrose in the tissues, others, that

[1] A preliminary communication of many of the observations included in this article appeared in the Zentralblatt für Physiologie, 1913 (March), p. 1311.

it participates in the control of the mobilization of dextrose from the liver. According to this latter view the disturbance which removal of the pancreas creates is one in which the liver not only loses the power of retaining absorbed dextrose as glycogen but in which it also develops in an exaggerated degree the power of producing sugar out of proteins and certain fats (gluconeogenesis). The sugar which thus passes the liver without having gone through a glycogen stage, and the new sugar which has been produced, accumulate in the blood more quickly than the tissues can utilize them, even although the tissues are not believed to show any less than their normal glycolytic powers.

A third view, which is really a compromise between the other two, supposes that the tissues cannot utilize dextrose until after it has undergone a preliminary transformation, one stage of which involves the production of glycogen for which the pancreatic hormone is necessary.

It has proved itself to be a most difficult matter to devise experiments which offer decisive evidence for or against any one of these hypotheses. Thus, the observations of Forschbach,[2] that the removal of the pancreas from one of two animals that had been previously united by sewing skin, muscles and peritoneum together (parabiosis) did not cause the usual degree of glycosuria, is unconvincing. Even if the observations themselves were free of criticism — which they are not — the immunity to diabetes of the depancreated animal might just as well be explained by local action of the pancreas of the anastomosed animal as by the presence of an internal secretion. The same criticism can be made of the observations of Carlson and Drennan[3] that pancreatectomy in pregnant dogs near full term is not followed by the usual degree of glycosuria. One of these authors (Drennan[4]) has furnished another type of evidence which if correct would certainly favor the "hormone" hypothesis. He found that the amount of sugar excreted by a depancreated dog in twenty-four hours became less when blood from a normal animal was intravenously injected. Unfortunately, however, the urine alone was examined;

[2] FORSCHBACH: Deutsches medicinische wochenschrift, 1908, xxxiv, p. 910; also Archiv für experimentelle Pathologie und Pharmacologie, 1908, ix, p. 131.

[3] CARLSON and DRENNAN: this Journal, 1911, xxviii, p. 391.

[4] *Ibid*, p. 396.

the evidence would be much more convincing if the behavior of the blood-sugar had also been ascertained.

Seemingly incontrovertible evidence in favor of the "hormone" hypothesis has recently been furnished by Knowlton and Starling.[5] These authors have studied the rate of dextrose consumption in blood perfused through the heart and lungs of normal and depancreated dogs. They found that the normal heart in an hour consumed about 4 mg. of dextrose for every gram of heart muscle, whereas the diabetic heart in four cases did not consume any dextrose in this time. They also found that the rate of disappearance, although low at first, gradually became greater when blood from a normal animal was perfused through a diabetic heart, and conversely, that the perfusion of blood from a diabetic animal through a normal heart was followed by a practically normal rate of dextrose consumption for the first hour but that this diminished subsequently. The interpretation which Knowlton and Starling put on these results is that the normal blood and tissues contain some substance which is necessary for the utilization of sugar in the organism, and they further offer, as evidence that the "hormone" is derived from the pancreas, the observation that the addition of a decoction of the pancreas, made with faintly acid Ringer's solution and subsequently neutralized with sodium carbonate, caused the diabetic blood to reacquire its normal glycolytic power, when perfused through the diabetic heart.

Confirmatory evidence for these remarkable observations has been furnished by Maclean and Smedley[6] who found that there was very much less consumption of dextrose from oxygenated Locke's solution when this was perfused through the heart of a depancreated animal than when it was perfused through a normal heart. These authors were however unable entirely to confirm Knowlton and Starling's statement that the addition of pancreatic extract restores the sugar-consuming power. They point out that the extracts may not have been prepared under exactly the same conditions.

Although the conclusions which are drawn, at least in so far as

[5] KNOWLTON and STARLING: Zentralblatt für Physiologie, 1912, xxvi, p. 169; Proceedings of the Royal Society, 1912, lxxxv, p. 218; The Journal of physiology, 1912, xlv, p. 146.

[6] MACLEAN and SMEDLEY: The Journal of physiology, 1913, xlv, p. 470.

they refer to utilization of sugar by the heart, do seem to be justified by the published results, there are, nevertheless, several technical details in connection with the experiments which demand attention.

In the first place, it is unfortunate that an untried method was employed by Knowlton and Starling for estimating the amount of sugar in the blood mixture that was used for perfusion. This method consisted in precipitating the proteins in the blood or serum with copper sulphate and subsequently removing the excess of copper in the protein-free filtrate with sodium hydrate. We have found, by comparison of results obtained on the same blood by this and the well-tried method of Rona and Michaelis, that considerable discrepancy is likely to occur unless very great care is taken to add just exactly the amount of sodium hydrate that is necessary to neutralize the cupric sulphate. Even with these precautions, we have been unable to obtain the same close agreement between the duplicates that is observed when the Rona-Michaelis method is employed, nor have we found that the results obtained by the two methods always agree with one another. These facts are shown in the following table:

TABLE I

SUGAR IN BLOOD OR SERUM AFTER REMOVAL OF THE PROTEIN BY COPPER SULPHATE OR COLLOIDAL IRON

Fluid employed	Reducing Substance in per cent after precipitation by:	
	$CuSO_4$	Colloidal iron
I. Dog blood	0.098 0.127	0.122 0.122
Dog serum	0.153 0.145	0.161 0.161
II. Dog serum and 0.2 [1] per cent dextrose	0.326 0.338	0.336 0.334
Dog serum and 0.4 [1] per cent dextrose	0.416 0.519	0.537 0.517
III. Ox blood and 0.4 [1] per cent dextrose	0.623 0.631	0.611 0.626
Serum of above	0.967	0.999

[1] These percentages are approximate.

We were never able to obtain accurate agreement between the duplicates by the copper method whereas this is usually the case when the colloidal method is employed. We have probably omitted some precaution which Knowlton and Starling adopted, but of which they do not warn us in their published papers. We can only add that we have been most careful to follow their directions. These authors themselves obtained much more satisfactory results; for example, out of eleven duplicate analyses there was absolute agreement in six, a difference of less than 2 per cent in three. In two cases, however, the error was more than 5 per cent.

It is stated by Knowlton and Starling that the amount of glycolysis in the blood under the conditions of their experiments could not have exceeded 0.01 per cent dextrose per hour. This is without doubt too low a figure. It is at least very much less than that found by one of us (J. J. R. M.) to apply in the case of defibrinated or "hirudin" blood of the dog incubated under strictly sterile conditions at body temperature;[7] thus, in defibrinated blood the following percentile glycolysis was observed:—40 (2 hrs. 15 min.), 55 (2 hrs. 50 min.), 34 (2 hrs. 30 min.), 27 ($1\frac{1}{2}$ hrs.); and in Hirudin blood these values were: 70 (2 hrs. 30 min.) and 37 (2 hrs. 30 min.). It should be pointed out, however, that in the experiments referred to no dextrose was added to the blood whereas in those of Knowlton and Starling a considerable quantity of dextrose was usually added. This was especially the case in those bloods which exhibited a very slight degree of glycolysis (*cf.* Table I of Knowlton and Starling). The importance of this observation will be discussed in a subsequent paper; meanwhile it is significant to note that the sugar content of the blood used for perfusing the heart of the diabetic dogs was very high in the three cases recorded in which no sugar disappeared, (Nos. 12, 17, and 18, Table III), possibly because dextrose had been added in excess of the amount usually present, even in diabetic blood.

Edelmann,[8] working with oxalate blood (of the dog) found that after two hours incubation from 11.26 to 24.24 per cent of sugar disappeared and Loeb,[9] using defibrinated blood, found in ninety minutes

[7] Unpublished experiments. The sterility of the blood was tested by bacteriological examination.

[8] EDELMANN: Biochemische Zeitschrift, 1912, xl, p. 314.

[9] LOEB, A.: *Ibid.*, 1913, xlix, p. 413.

that from 48 to 62 per cent disappeared. The former author also found that a certain amount of glycolysis likewise occurs in diabetic blood and this was also observed to be the case by Knowlton and Starling.[10] It is somewhat difficult to harmonize this fact with the statement that in blood perfused through the diabetic heart there should sometimes be no glycolysis whatsoever.

Although, as computed from Knowlton and Starling's tables, it is the case that the general average for sugar consumption by the normal hearts was about 4 mg. per gram heart muscle per hour yet there were very great deviations from this average; thus, leaving out of account cases in which the anaesthetic was left on by mistake, or in which the blood was very venous, the variations ran from 2.84 to 6.29 mg. per gram muscle per hour. In three of the seven hearts of diabetic animals the consumption varied from 1.8 mg. to 4.9 mg. per gram per hour, but this almost normal consumption is explained by the authors as probably due in part, at least, to bacterial growth; in the remaining four observations of this series extra precautions against bacterial growth were taken and no dextrose was used. In five diabetic dogs of another series of observations the consumption was much less than normal.

It is stated by Knowlton and Starling that the diabetic heart beat was very slow but that it increased in rate whenever pancreatic extract was added to the perfusion fluid. This and the accompanying partial restoration in sugar consuming power may have depended on the fact that the extract was neutralized with sodium carbonate, the presence of which in Locke's solution as Neukirch and Rona [11] have shown materially augments the beat and increases the sugar consuming power of the heart. Knowlton and Starling also observed slight quickening of a perfused diabetic heart when some sodium bicarbonate was added to the perfusion fluid.

In making these criticisms we do not desire to be understood as denying the possibility that less sugar may be used by the diabetic as compared with the normal heart. We believe however that the observations so far recorded do not unmistakably prove this fact.

[10] KNOWLTON and STARLING: *Cf.* Proceedings of the Royal Society, 1912, B., lxxxv, p. 221.

[11] NEUKIRCH and RONA: Archiv für die gesammte Physiologie, 1912, cxlviii, p. 285.

But even if the diabetic heart should consume less sugar it is not justifiable to conclude that removal of the pancreas brings about the disappearance from the blood of some substance which is necessary for the utilization of carbohydrates in the other tissues of the body. It is necessary before drawing such a conclusion to compare the sugar utilization in the skeletal muscles of normal and diabetic animals. Theoretically, this could most simply be done by ascertaining the rate of sugar consumption in the artificially perfused hind limbs. Since such experiments always involve a certain risk of bacterial contamination, and since there are other technical difficulties and sources of inaccuracy connected with them, we have put the question to the test by observing the behavior of the sugar in the blood of dogs from which all the abdominal viscera were removed. Following such evisceration, as Bock and Hofmann, Pavy, and one of ourselves [12] have shown, the percentage of sugar in the blood steadily falls because the utilization of sugar in the tissues cannot be compensated by an increased discharge of sugar from the liver. Such preparations may really be considered as perfusions of the muscles with blood pumped by the heart and arterialized by the lungs. In them the conditions are certainly more nearly normal than is the case when an artificial pump and *in vitro* arterialization of the blood are employed. There are however two difficulties which present themselves in the use of such preparations. The first of these is that anaesthetic must continue to be administered, the presence of which in the blood might, as Knowlton and Starling imply, depress the glycolytic power. To control this, in about one half of our experiments, we have tied the innominate and left subclavian arteries just after their origin from the aorta, thus removing the higher nerve centres from the circulation and rendering the administration of anaesthetic unnecessary. As can be seen from the tables of results, however, this did not measurably affect the rate with which sugar disappeared. The other difficulty was with regard to the arterial blood pressure. Although this invariably rose considerably, as an immediate result of the ligation of the coeliac axis, it subsequently fell, as a rule, until, in some cases, it came to be no higher than about 40 mm. Hg; indeed, especi-

[12] Bock and Hofmann: Experimental Studien über Diabetes, Berlin; Pavy and Siau: The Journal of physiology, 1903, xxix, p. 375; Macleod: this Journal, 1909, xxiii, p. 278.

ally in our earlier observations, the blood pressure might steadily decline to zero within a period of about half an hour after ligation of the abdominal vessels.

The cause for the fall of pressure in these earlier experiments was probably hæmorrhage into some untied small branch of the aorta, for the viscera were not actually removed after ligation of the vessels. This bleeding may have been through vessels coming to the stomach from the oesophagus or on to the rectum from the inferior haemorrhoidal arteries. In the subsequent experiments, where actual evisceration was practiced, these vessels were necessarily tied.[13] But even when every precaution was taken to avoid any such leakage of blood into untied splanchnic vessels, the blood pressure usually fell, so that within an hour after the evisceration it was no more than 60 mm. Hg. Such a fall was not experienced by Pavy. Since we have found from experience that irregular results in the amount of sugar in the blood are likely to be obtained when the blood pressure is much below 40 mm. Hg., we have, in the later experiments of the present research, kept it at a higher level than this by intravenous injections of adrenalin. The amount of adrenalin injected was always adjusted so as to keep the pressure as constant as possible. The preparation used was 1–1000 adrenalin chloride (Parke Davis); in some cases it was injected undiluted, in others it was diluted five times with Locke's solution. The amounts required were never large and even in the cases where the dilute solution was employed were never sufficient to bring about any material dilution of the blood. It can be seen, by comparing the results obtained in cases with and without adrenalin, that the injection did not in any way influence the rate of sugar disappearance. In certain of the experiments the animals died from cardiac failure in spite of all we could do to prevent it and we were compelled to take the blood for analysis from the heart chambers after death. We have found, however, that the amount of sugar in such blood is practically always considerably in excess of that present in blood removed even a few minutes previously from the renal vein or carotid artery.

The sugar was estimated in the blood by Bertrand's method after

[13] On account of Pavy's observation that even after ligation of the portal vein there may be some sugar discharged by way of the hepatic veins, we have in all experiments applied mass ligatures to the back portions of the liver lobes.

removal of the proteins of means of colloidal iron. In as many cases as possible duplicate analyses were made.

Consideration of Results

In order that we might have some standard with which to compare the rate of glycolysis following pancreatectomy, a series of observations were made on samples of blood removed at varying intervals after evisceration in normal dogs. It was hoped that it would be possible so to control the conditions of the experiments, that a constant rate of sugar disappearance for different animals could be determined. The results actually obtained in a series of eleven such observations are given in Table II from which it will be seen that no such constancy was attained. The values set down in the fourth column of the table most clearly demonstrate the rate of glycolysis. They represent the milligrams of dextrose which disappeared from 100 gm. of blood during one minute.

TABLE II

Glycolysis in the Blood of Eviscerated non-diabetic Dogs

No.	Time after evisceration (minutes)	Dextrose in blood (per cent)	Amount of dextrose disappearing from 100 gm. blood per minute (milligrams)	Remarks	
1	15 33 60	0.112 0.097 0.088	0.83 0.33	Ether anaesthesia. B. P. fell from 120 to 40 mm. Hg.	Abdominal vessels ligated (no adrenalin)
2	15 30 45	0.244 0.208 0.190*	2.4	Morphine and urethane. B. P. fell from 70 to 10 mm. Hg. * Dead.	
3	15	0.185 0.164	1.4	Cerebral vessels tied. B. P. fell from 50 to 10 mm. Hg.	
4	15	0.113 0.100	0.86	Ether anaesthesia. B. P. 50 mm. Hg. falling to zero.	

TABLE II (*Continued*)

No.	Time after evisceration (minutes)	Dextrose in blood (per cent)	Amount of dextrose disappearing from 100 gm. blood per minute (milligrams)	Remarks	
5	30 45 60	0.178 0.156 0.142	1.46 0.93	Ether and urethane.	
6	0† 24 39	0.287 0.223 0.162*	2.66	Morphine and urethane. B. P. 90–40 mm. Hg. * Heart-blood. † 10 min. after operations.	Abdominal vessels ligated and defibrinated blood *plus* dextrose then injected. (No adrenalin)
7	0† 30 45 60	0.195 0.172 0.165 0.175*	1.53 0.46	Ether and urethane. B. P. 120–20 mm. Hg. * B. P. practically zero. † 15 mm. after operations.	
8	0 15 45	0.229 0.162 0.133	4.46 0.97	Morphine and urethane. B. P. 80–20 mm. Hg.	
9	0 15 30 45	0.341 0.299 0.272 0.245	2.8 1.46 1.80	Ether anesthesia. B. P. [illegible]–60 mm. Hg. (Viscera not removed), 1–5000 adrenalin.	
10	15 30 45	0.113 0.096 0.073	1.10 1.40	Cerebral vessels ligated, 75 c.c. 1–5000 adrenalin. B. P. 80–40 mm. Hg. Starved animal.	Abdominal vessels ligated and viscera removed in two experiments. Adrenalin injected.
11	0 30 60	0.085 (0.082) 0.057	1.16	Hemorrhage during operations, only 1 c.c. 1–1000 adrenalin used. B. P. 60 mm. Hg.	

In the first four experiments no special precautions were taken to prevent the fall of blood pressure ensuing upon evisceration; and if we leave out of account those determinations which were made on blood samples removed when the blood pressure was near zero,

TABLE III

GLYCOLYSIS IN THE BLOOD OF EVISCERATED DIABETIC DOGS

No.	D. N. ratio	Time after evisceration (minutes)	Dextrose in blood (per cent)	Amount of dextrose disappearing from 100 gm. blood per minute (milligrams)	Days after pancreatectomy	Remarks
3	4.6	0 30 45	0.231 0.145 0.113	 2.86 2.10	3	Cerebral vessels ligated; 40 c.c. 1–1000 adrenalin injected. B. P. 80–40 mm. Hg.
4	2.8 3.3 (starvation)	0 15	0.305 0.235	4.60	5	Ether anaesthesia. No adrenalin. Liver lobes not ligated. Viscera not removed. B. P. 80–40 mm. Hg. Dog almost moribund before experiment started.
8	3.3	15 45 60	0.311 0.180 0.147 } 0.147 }	 4.3 2.2	3	Cerebral vessels ligated; 30 c.c. 1–5000 adrenalin. B. P. 120–80 mm. Hg. During experiment cardiac failure with resuscitation.
9	2.8	0 20 35	0.425 0.385 (0.164)	2.0	4	Cerebral vessels ligated 75 c.c. 1–5000 adrenalin.
10	4. 4.4 (some milk allowed)	0 30 60 90	0.303 0.288 0.273 } 0.268 } 0.249	 0.5 0.6 0.7	5	Ether anaesthesia. B. P. remained above 60 mm. Hg. up to 90 mm., after which 1–5000 adrenalin injected to maintain B. P. One kidney not removed.

		120*	0.232 0.210) 0.213)	0.56		* Normal blood *plus* dextrose injected. B. P. now averaged 140 mm. Hg.
		27	0.160) 0.172)	1.66		
11	3.9 (starvation)	0 30 60 90	0.292 0.270 0.224 0.202) * 0.193)	0.73 1.53 0.91	6	Ether anaesthesia. Both kidneys ligated; after 60 min. 2 c.c. 1–1000 adrenalin injected slowly. B. P. 90–60 mm. Hg. * Heart blood.
12	3.8 (starvation)	0 40 53	0.331 0.222 0.144*	2.72	6	Ether anaesthesia; 100 c.c. 1–5000 adrenalin injected. Both kidneys ligated. B. P. 80–40 mm. Hg. * Heart blood.
13	— (haematuria)	0	0.277) 0.264)	3.7	7	Ether anaesthesia. Both kidneys ligated; 35 c.c. 1–5000 adrenalin injected. B. P. 60–20 mm.
		30 45	0.157) 0.157) 0.130	1.8		
14	1.8	0 30 38	0.269 0.226 0.341*	1.43	4	Cerebral vessels ligated; less than 5 c.c. 1–1000 adrenalin. Both kidneys ligated. B. P. 120 mm. gradually falling. * Collected from heart after death.
15	3.57	0 30 60 90	0.265 0.220 0.138 0.136*	1.50 2.73	5	Cerebral vessels ligated. Both kidneys ligated; 40 c.c. 1–5000 adrenalin mostly after 60 min. B. P. 80 mm. Hg. until after 60 min., when it began to fall. * Animal just dead.

it will be seen the dextrose consumption varied between 0.83 to 2.4 mg. In the next four experiments (Nos. 5 to 8 inclusive) it was attempted to maintain the blood pressure in the eviscerated animals by removing usually about 150 c.c. of blood from the animal at the start of the experiment and after defibrination and the addition of an equal volume of Locke's solution, containing 1 per cent dextrose, reinjecting it into the animal immediately after the evisceration. This was done in order to leave as small a quantity of blood as possible in the tied-off splanchnic vessels. The procedure did not materially prevent the rapid fall of blood pressure. The glycolysis was naturally somewhat quicker immediately after the injection (in one case rising to 4.46 mg.) but after some time it fell to between 0.46 and 0.97 mg. dextrose per minute.

In the last three experiments of the table, adrenalin (1–1000 or 1–5000 in Locke's solution) was injected at constant pressure into the renal vein at such a rate as to keep the blood pressure at least above 60 mm.; the dextrose consumption varied between 1.13 and 2.8 mg. per minute, thus indicating, when compared with the previous figures, that the adrenalin had no material influence on the rate of glycolysis.

Under the conditions of these experiments there is therefore a considerable variation in the glycolysis of different normal animals and it is not possible to co-relate the variations either with the mean arterial blood pressure or with the tying off of the head arteries which of course also involves opening the thorax and applying artificial respiration.

Turning now to the results obtained on diabetic animals, which are given in Table III, it is to be noted that in a much larger proportion of the experiments the fall of blood pressure produced by evisceration was compensated by injections of adrenalin. These experiments, being on very valuable material, were naturally not undertaken until after the experience gained by work or normal animals had been obtained, the outcome of which as we have seen was to indicate adrenalin injections as the most satisfactory means of keeping up the blood pressure That such injections did not bear any relationship to the rate of glycolysis in diabetic animals was demonstrated in one or two cases in which the drug did not require to be given until the latter part of the experiment, in one case (No. 10) the rate of glycolysis was the same before and after the injection,

in another (No. 11) it was less and in a third (No. 15) it was greater. Nor was ligation of the vessels proceeding to the head and fore limbs associated with any demonstrable variation in the glycolysis. Thus, in the cases in which these vessels were untied (Nos. 10, 11, 12, 13 and 14), and the anaesthesia consequently maintained the rate of glycolysis varied between 0.5 (No. 10) and 4.6 (No. 4) mg. per minute, whereas in those in which the vessels were tied (Nos. 3, 8, 9, 14 and 15), it varied between 1.5 and 4.3 mg. per minute.

When we compare the results obtained on diabetic with those obtained on normal dogs it is plain that no difference can be made out. For some reason which we cannot explain, the results in both cases are extremely variable and it, therefore, becomes impossible to give any average which would be at all reliable for either series of observations. However, some comparisons may be of interest. The average dextrose consumption for the 11 normal dogs (17 observations) is 1.63 mg. per minute and for the 10 diabetic animals (19 observations) it is 1.86 mg. per minute.

The maximum and minimum rates for the non-diabetic animals (including those in which dextrose was injected) are 2.4 and 0.83 mg. per minute respectively, and for those that were diabetic, 3.7 and 0.50.

In one experiment on a diabetic animal (No. 10) there does appear to be distinctly less glycolysis than in any other case, either normal or diabetic. This was an unusally resistant, although markedly diabetic, animal, the blood pressure remaining above 60 mm. for more than an hour after the evisceration so that no adrenalin had to be injected until after ninety minutes. The dextrose consumption meanwhile ranged between 0.5 and 0.7 mg. per cent per minute. Unfortunately in this experiment the kidney on the right side was not tied off so that the comparatively small decrease in dextrose which did occur might be accounted for, in part at least, by excretion into the urine. In five other experiments, however, the vessels of both kidneys were ligated and in those the glycolysis was very marked. The injection of 250 c.c. defibrinated blood containing 5 per cent dextrose into this dog was followed by more marked glycolysis which might be attributed to the influence of pancreatic hormone. We are not prepared to give any other explanation at present, only we would point out the remarkable rate in the experiments on normal dogs, in which dextrose was injected, at which it disappeared. Perhaps, the dextrose

introduced in this way finds some depôt other than the liver in which it is converted to glycogen. The possibility of its being retained in the lymph must also be borne in mind.

We cannot draw any conclusions from our results regarding the comparative rates of sugar utilization by the normal and the diabetic heart, but we believe that there is no difference in this regard in so far as the skeletal muscles are concerned. It is possible that the difference observed by Knowlton and Starling in sugar consumption between normal and diabetic hearts might have existed under the conditions of our experiments and yet have been insufficient to make an impression on the blood of the eviscerated animal. The conditions which influence the rate of sugar disappearance are so variable in different animals, and even in the same animal at different periods of the same observation, that it has proved impossible to obtain an average figure for sugar utilization by the use of which we could decide whether this was more marked in the one group than in the other. As already pointed out we can offer no explanation for these variations. We are certain, however, that they do not depend on any error in the blood-sugar estimations.

As a criterion of the degree of diabetes in the depancreated animals we have as usual taken the D. N. ratio. In one instance this could not be obtained because of an unaccountable and severe haematuria. Most of the animals received a pint of milk for the day or two following the pancreatectomy and meat on the succeeding days. In one or two instances they were starved for the day immediately preceding the evisceration experiment. It will be seen from the second column that all of the dogs were diabetic to the full degree. Although the operations were performed with every aseptic precaution possible and were kept afterwards in thoroughly clean cages there was always some suppuration of the wound, a condition which we have very rarely experienced in operations on non-diabetic animals. For example, in the experiments numbered three and four in the table the pancreatectomy was performed in two stages; in the first one the gland was removed except for the free vertical portion (processus uncinatus) which was grafted, with its blood vessels intact, in the subcutaneous tissues of the abdominal wall. After this operation there was no suppuration. The graft was then removed (without opening the abdomen) and the wound suppurated.

In conclusion we may point out that even if the isolated heart of the diabetic animal should be unable to utilize dextrose, this need not necessarily imply that it is because of the absence of some hormone which is necessary for utilization of dextrose by the heart muscle. It may be because of the presence in diabetic blood of toxic substances which may interfere with sugar utilization by the heart, under the conditions of the perfusion experiments. The fact that addition of pancreatic extract to the diabetic blood brings about a restoration of the glycolytic power — a fact which is not, we believe, supported by a sufficient amount of evidence (see p. 186) — would certainly offer stronger support to the hormone hypothesis but it would not necessarily render the "toxic" hypothesis untenable, for such an extract might contain antitoxin.

ON THE FORMATION OF FAT FROM CARBOHYDRATES

By SERGIUS MORGULIS and JOSEPH H. PRATT

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THE experiments described in this paper have all been performed on a dog belonging to one of us (J. H. P.), which showed certain peculiarities in the metabolic activity due to pancreatic achylia.

On March 29, nearly eight months before our experiments were begun, the corpus pancreatis of the dog was removed, leaving the processus lienalis and the processus uncinatus in situ. A detailed description of the operation and subsequent history of this dog "Flora" is given in another paper.[1]

At the time of the operation the dog was very fat and weighed 13.8 kg. There was a steady loss of weight from the time of the exclusion of the pancreatic juice from the intestine to the end of June, when it had fallen to 6.5 kg. From this time to the middle of September the weight was fairly constant. Diabetes did not develop. The administration of pigs' pancreas, which was begun in September, was followed by a gain in weight. When the feeding of pancreas was discontinued on October 21 the dog weighed 8.35 kg.

An absorption experiment was begun on November 12 and continued for four days. During this period the dog ate 3000 gm. of meat. 42.23 per cent of the nitrogen of the food was lost in the feces and 72.73 per cent of the fat. The animal was in nitrogenous equilibrium, losing only .02 gm. of body nitrogen during the four days.

The experiments were performed at the Nutrition Laboratory with the dog apparatus constructed on the principle of the closed circuit, and essentially like that described by Prof. F. G. Benedict

[1] Benedict, F. G., and Pratt, J. H.: Journal of biological chemistry, July, 1913.

in an earlier paper [2] but modified by him for direct determinations of oxygen. It would be superfluous to give here a detailed description of the apparatus; it will suffice to mention that the animal's muscular activity has been carefully controlled by means of a pneumograph attached to the cage in which the animal is kept, and communicating with a Marey tambour. The cage being suspended by a spring at one end and resting on two knife edges at the opposite end is sensitive to the slightest movement on the part of the animal, and the movements are recorded on a kymograph by means of a pointer resting on the tambour. The carbon dioxide, absorbed in soda lime bottles, is ascertained by the difference in weight of the bottles at the beginning and end of each experiment. As there are two sets of soda lime absorbers attached to the apparatus, either one or the other may be brought into operation by a shift of a three-way valve. By this arrangement it was possible to determine the carbon-dioxide production during successive half-hour periods without interrupting the course of the experiment. The oxygen was admitted into the system from a small cylinder, which was likewise weighed before and after each period. The amount of oxygen thus determined by weight was also checked by readings of a Bohr meter through which the oxygen passed before entering into the system. The relative humidity of the air of the respiration chamber was measured by a psychrometer; samples of the air at the end of the experiment or at the end of each successive period have been analyzed for carbon dioxide with the Sondén apparatus.

Knowing the empirical volume of the entire system (including the chamber and accessories) we were able to compute the quantitative composition of the residual air at the end of each period by making use of the data obtained from readings of the psychrometer, barometer, thermometer, and the per cent of carbon dioxide in the air. The difference in the residuals enabled us to make corrections for the consumption of oxygen and the production of carbon dioxide as determined directly by weight.

The efficiency and accuracy of the respiration apparatus have been frequently tested, and we did not run regular experiments before complete satisfaction as to its perfection in this regard could be

[2] Benedict, F. G., and Homans, J.: this Journal, 1911, xxviii, p. 29; Journal of medical research, 1912, xxv, p. 409.

obtained. The system was tested for tightness and blank experiments showed no changes in the weight of the soda-lime absorbers. Furthermore, check experiments were performed from time to time by burning ethyl alcohol in the respiration chamber. Almost invariably the quotients obtained were in complete agreement with the theoretical expectation for the combustion of alcohol, thus giving positive proof of the accuracy of our apparatus.

There is nothing essentially novel in the fact that within the organism of an animal one substance may be chemically transformed into another substance. The formation of carbohydrates from fat or protein, and the formation of fat from carbohydrates have been maintained by physiologists on different occasions, and in the majority of cases with sufficient justification. The transformation of fat into carbohydrates, for instance, figured greatly in metabolism studies with hibernating animals where it was thought that this transformation furnished the raison-d'être for the extremely low respiratory quotients observed by several investigators. This hypothesis, however, has serious objections against it, and recently it has been severely criticized by Nagai,[3] who showed that with accurate and well-controlled methods one does not find quotients with hibernating animals as low as were hitherto claimed. The fact of the transformation of carbohydrates into fat, on the contrary, has been receiving more and more confirmation ever since Liebig supposed that such a change may occur, and it may be said that today this is fairly beyond questioning. It was corroborated by the experience of breeders and agriculturists that rich carbohydrate food is conducive to the fattening of the stock. Henneberg (1881),[4] Chaniewski (1884),[5] Munk (1885),[6] Meissl (1886)[7] and others[8] experimenting with various animals, such as sheep, geese, dogs, pigs, found that in striking the balance of the intake and output, and comparing it with the actual acquisi-

[3] Nagai, H.: Zeitschrift für allgemeine Physiologie, 1909, ix, pp. 243–367.

[4] Henneberg, W.: Zeitschrift für Biologie, 1881, xvii, pp. 295–350.

[5] Chaniewski, T.: Zeitschrift für Biologie, 1884, xx, pp. 178–192.

[6] Munk, J.: Virchows Archiv für Pathologische Anatomie, 1885, cx, pp. 91–134.

[7] Meissl, E.: Zeitschrift für Biologie, 1886, xxii, pp. 63–160.

[8] Lehmann, K. B., und Voit, E. Zeitschrift für Biologie, 1901, xlii, pp. 619–671.

[8] Rubner, Max: Zeitschrift für Biologie, 1886, xxii, pp. 272–280.

tions of the body they could not evade the conclusion that the carbohydrates had contributed to the deposit of fat. These experiments dealt with the assimilation of protein, fat and carbohydrate unassisted by a parallel investigation of the respiratory exchange of the animals under observation. Yet, if the transformation of carbohydrate into fat is an actuality, it should be reasonable to expect some clue as to its occurrence in the gaseous metabolism, since a substance rich in oxygen is thereby changed into one poor in oxygen. In other words this process should become revealed in the respiratory quotient which is the ratio between the carbon dioxide produced and the oxygen consumed during any length of time.

When this paper was already written we found an interesting paper in Russian, in which the metabolism of both dogs and rabbits fed only on sugar was discussed. The author of this article apparently did not realize the significance of the respiratory quotient, as throughout his voluminous tables and text he makes no mention of it. He determined, however, the carbon dioxide production and oxygen consumption of his subjects, and we were thus able to work out the respiratory quotients from his data. The experiments were per-

TABLE I

Original data				Computed		
Dog	Days	CO_2	O	CO_2	O	Respiratory Quotient
		Average in grams per day		Average in litres per day		
Damka	9 fasting	265.1	224.7	134.96	157.29	.858
	9 sugar diet	389.2	223.4	198.14	156.38	1.267
	29 sugar diet	1094.8	747.6	557.36	523.32	1.065
Bijka	9 fasting	330.7	325.5	168.36	227.85	.739
	9 sugar diet	368.5	267.2	187.60	187.04	1.003

formed in the following way: An animal was first subjected to a fast lasting several days, with water, and the urine was analyzed for nitrogen and the various inorganic constituents. Then the animal, after it had recuperated from the fast and attained the initial weight again, was put on a diet of sugar either in the form of solid lumps or in the form of a solution which was given through the stomach tube. The urines were also collected and analyzed as in the preliminary period. In a few instances the gaseous exchange was likewise determined, and we used these data in compiling Table I.

We may observe that in either case with an exclusive sugar diet the dog had a respiratory quotient of over one. In the case of the dog "Damka" the average quotient for the first nine days of the sugar feeding was 1.267, and for the entire experimental period of twenty-nine days, 1.065. The other dog "Bijka" shows only a slight rise of the respiratory quotient over one, but the respiratory quotient of the first dog is likewise a great deal higher when fasting. It is possible that the method for measuring the gaseous exchange employed by Protasov [9] was not unimpeachable, as it is very improbable for an animal fasting nine days to have a respiratory quotient of .858, and it may be that the figures for the oxygen are lower than the actual consumption.

We believe that Pflüger first realized the importance of the gaseous exchange and of the respiratory quotient in the transformation of carbohydrate into fat when he instructed his student Bleibtreu [10] to carry out a study of the gaseous metabolism. Bleibtreu employed young geese which he fed superabundantly upon rye meal and found large deposits of fat in their bodies when they had been killed at the close of the experiment. He performed also several respiration experiments with the result, as was to be expected on theoretical grounds, that in each case the respiratory quotients rose above one, ranging from 1.117 to 1.38, thus furnishing new proof for the origin of fat from carbohydrates. While Bleibtreu's experiments are extremely interesting, and undoubtedly conclusive, yet owing to deficiencies of

[9] PROTASOV, I. I.: Metabolism of matter under condition of exclusive feeding with sugar. Dissertation, Imperial Military Medical Academy in St. Petersburg, 1895, p. 67. (In Russian.)

[10] BLEIBTREU, M.: Archiv für die gesammte Physiologie, 1901, lxxxv, pp. 345–400.

his apparatus they leave room for criticism, and it seems to us not unlikely that with methods more properly controlled one would not find quotients as high as 1.38. However this may be, his results are true in the main and the quotients of over one may be taken as a direct proof of the phenomenon which has been recognized and postulated long ago.

Although the point may be considered well established, we do not hesitate to contribute to this subject as the conditions under which the transformation of carbohydrates into fat took place are rather singular. Besides, our experiments present practically the first series of determinations of oxygen and carbon dioxide coincident with this transformation (certainly in the case of the dog) which have been made with a well-controlled and critical method. Our dog was in a very emaciated state and since, as was stated above, she could utilize but a fraction of the protein and fat in the ingested meat, most of it (40 and 70 per cent respectively) being excreted in the form of large bulky stools, she was fed on glucose besides, and on one occasion we observed that the respiratory quotient increased above one. Suspecting that our dog was forming fat, we decided to follow up this matter.

We proceeded to feed the dog large quantities of glucose, expecting by thus over-feeding her with carbohydrates to find a state resembling somewhat that observed by Bleibtreu. The dog was given daily at least 120 gm. of glucose with some meat, and on the days on which respiration experiments were performed she got as much as 200 to 225 gm., with a relatively small admixture of chopped meat. It is interesting to note that in spite of such an abundance of nourishment her body weight remained practically unchanged throughout the experimental period of nearly three weeks.

Having given the dog glucose in the food for two preceding days, she was brought into the respiration chamber on November 18, two hours after eating 100 gm. of glucose and 300 gm. of meat. The dog remained in the chamber for two hours and during that time she produced per hour 4.12 litres of carbon dioxide and consumed 3.90 litres of oxygen. A week later the dog was fed 300 gm. of meat and 125 gm. of glucose and about three hours later a similar amount of meat and 100 gm. of glucose, after which she was placed in the respiration apparatus for two hours. This time she produced per hour 4.96

litres of carbon dioxide and consumed 4.78 litres of oxygen. The same thing was repeated next day. The animal received 50 gm. of meat and 100 gm. of glucose in the morning, then three hours later another portion of 50 gm. of meat and 125 gm. of glucose, and went directly into the respiration chamber. The dog produced per hour 5.40 litres of carbon dioxide and consumed 5.19 litres of oxgyen. On November 26, the dog was again given 50 gm. of meat and 125 gm. of glucose, and a respiration experiment lasting nearly two hours was immediately begun. Then she received another 100 gm. of meat and 100 gm. of glucose and the interrupted experiment was continued for another two hours. During these two successive experiments the animal produced per hour 4.20 and 4.50 litres of carbon dioxide and consumed 3.92 and 4.22 litres of oxygen respectively, but the respiratory quotients in both experiments were practically the same. The last experiment of the series was performed a week later during which time the dog was fed on meat and glucose. On the day of the experiment she received 50 gm. of meat and 125 gm. of glucose, but could not be induced to eat any more, and vomited at the mere sight of food. Under these circumstances we were obliged to resort to injecting the glucose subcutaneously, and we thus introduced into the body 240 c.c. of a 20 per cent aqueous solution of glucose, making a total of 48 gm. of glucose. The dog was put directly into the respiration chamber and this time the highest respiratory quotient of practically 1.1 was obtained. The respiratory quotients obtained in this series of experiments range from 1.038 to 1.099 and the data are presented in tabular form in Table II.

Since the respiratory quotient is the ratio between the carbon dioxide production and the oxygen consumption, the high quotient contingent upon the formation of fat from carbohydrate may be either due to an increase of the numerator, or to a decrease of the denominator; the numerical outcome in both events remains the same. The latter course is in agreement with Liebig's conception of this transformation process. Pflüger's idea of an intramolecular migration of the oxygen atoms resulting in a formation of carbon chains of which the fat molecule is afterwards reconstructed, is probably the more correct one. The postulate that the transformation is accompanied by an increased output of carbon dioxide which is to be expected on this assumption, finds corroboration at least in some of our experi-

ments. Thus we observed a gradual rise in the carbon dioxide production in successive periods of thirty minutes each while the oxygen consumption remained practically constant. In one of the experiments the dog having been placed in the respiration chamber two

TABLE II

Date	Body weight in kilograms	Diet	Carbon dioxide production	Oxygen consumption	Respiratory quotient
			per hour		
Nov. 18	6.08	9.30 A.M. 300 gm. Meat + 100 gm. Glucose	4.12	3.90	1.055
Nov. 25	6.50	11.30 A.M. 300 gm. Meat + 125 gm. Glucose	...	...	....
		3.00 P.M. 300 gm. Meat + 100 gm. Glucose	4.96	4.78	1.038
Nov. 26	6.60	11.00 A.M. 50 gm. Meat + 100 gm. Glucose	...	...	....
		2.00 P.M. 50 gm. Meat + 125 gm. Glucose	5.40	5.19	1.040
Nov. 27	6.68	9.00 A.M. 50 gm. Meat + 125 gm. Glucose	4.20	3.92	1.073
		11.00 A.M. 50 gm. Meat + 100 gm. Glucose	4.50	4.22	1.058
Dec. 3		9.45 A.M. 50 gm. Meat + 125 gm. Glucose	...	...	....
		3.00 P.M. Injected subcutaneously 240 c.c. of a 20 per cent solution of Glucose	5.51	5.01	1.099
		Average	4.78	4.50	1.062

hours after a meal of meat and glucose the carbon dioxide output was continually increasing from 1.88 to 2.21 litres for thirty minutes. In another experiment tabulated below, the carbon dioxide output was gradually increasing from 2.25 to 2.65 litres in successive thirty-minute periods, but the oxygen consumption, if we take into consideration that the amounts for the second and third periods probably

compensate for each other, remains practically 2.39 in each period. Although in the early part of the fourth period there was some muscular activity, the dog has been very quiet in the second and third periods, as can be seen on the kymograph records, while she was less quiet in the first period. This must be taken as good evidence that the increasing output of carbon dioxide has not been caused by an increased muscular activity of the subject.

TABLE III

Date	Carbon dioxide (per hour)	Oxygen (per hour)	Respiratory quotient	Diet
Nov. 25, 1912				
3.30 — 4.00 P.M.	2.25 l.	2.39 l.	.941	11.30 A.M., 300 gm. Meat +
4.00 — 4.30 P.M.	2.41 l.	2.19 } 2.39 l.	1.100	125 gm. Glucose
4.30 — 5.00 P.M.	2.61 l.	2.58 }	1.012	3.00 P.M., 300 gm. Meat + 100
5.00 — 5.30 P.M.	2.65 l.	2.40 l.	1.104	gm. Glucose
Total for 2 hours	9.92 l.	9.56 l.	1.038	

It would be an extremely difficult task to figure out with some degree of accuracy the amount of fat which is formed from carbohydrates during the experimental period. Furthermore, as we did not analyze the urine excreted during a sufficient number of the respiration experiments, we lack the most important data for this purpose. Without pretending to estimate with any degree of precision the quantity thus formed, we may attempt to compute that amount approximately by making the justifiable assumption that the animal has been burning carbohydrates for its maintenance during the experiment. Following this line of reasoning, we should expect the carbon dioxide output to be equal to the oxygen intake during the same experiment. The excess of carbon dioxide set free in the chemical process of transformation of carbohydrates into fat is not a measure of the metabolic activity of the organism, nor is it connected with the production of heat. It is an extra quantity superimposed upon

the carbon dioxide that results from the maintenance combustion of the metabolism. Theoretically, we know that 2.7 gm. of glucose may give 1 gm. of fat with the liberation of .55 gm. of water, and 1.16 gm. of carbon dioxide. Hence it follows that for every gram of fat originating from carbohydrates there should be set free 1.16 gm. or .59 litre of carbon dioxide above the amount resulting from the combustion of the body materials. If we avail ourselves of Hanriot's formula for the transformation of carbohydrates into fat, where a hypothetic substance (stearo-oleo-palmitin) is imagined to be formed by satisfying the three valencies of a glycerid with the fatty acid radicles of the three chief representatives of animal fatty acids (stearic, palmitic, oleic acids), we would expect instead .60 litre of carbon dioxide. On the line of reasoning suggested above this extra amount of carbon dioxide resulting from the formation of fat can be computed by subtracting from the ascertained quantity of carbon dioxide an amount equal to that of consumed oxygen, which on the above supposition must have been directly produced in the process of combustion of carbohydrates. For the sake of convenience, we may make use of the average figures given in Table II which may serve as representative for the entire set of experiments recorded therein. We will observe that there were 4.78 litres of carbon dioxide produced and 4.50 litres of oxygen consumed per hour, and that the average respiratory quotient was 1.062. If carbohydrates alone had been burned for maintenance during that time 4.50 litres of carbon dioxide would have been derived from that source. The excess of .28 litre (4.78–4.50) of carbon dioxide is what on this view has been set free in the transformatory process which was going on simultaneously with the other. The theoretical expectation of the liberation of about .60 litre of carbon dioxide when one gram of fat is newly formed from glucose, is equivalent to a rise in the respiratory quotient by .131 when the animal's consumption of oxygen per hour is 4.50 litres, as may be gotten by dividing .60 by 4.50. The average respiratory quotient observed in our experiments is .062 above one, or roughly one-half of the theoretically expected rise of the quotient, when one gram of fat is newly formed. In other words, we may assume that in the course of one hour .5 gm. of fat are being formed from carbohydrates on the average in such an extreme case as when carbohydrates alone are being burned for maintenance. As a matter of fact, how-

ever, the experiments with our dog have shown that on a mixed diet, though the respiratory quotient was very high, it never reached unity, but was usually about 0.940. We may, therefore, assume further that under the experimental conditions and the condition of the dog described here, the animal deposited on an average a gram of fat per hour.

In concluding we wish once more to emphasize the fact that a weak and emaciated dog with severe disturbance in the absorption of fat and protein was still able to form fat from carbohydrates. Apart from the additional proof which our investigation brings to the theory of the transformation of carbohydrates into fat, it also shows that the transformation may and actually does take place even in the carnivorous dog.

THE ACTION OF THROMBOPLASTIC SUBSTANCE IN THE CLOTTING OF BLOOD

By F. W. MacRAE, Jr. and A. G. SCHNACK

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IN the theory of the coagulation of the blood which we owe to Morawitz it is assumed that the prothrombin is converted to active thrombin by the combined influence of calcium and thrombokinase, the latter element being furnished by the tissue cells, including the blood corpuscles. Howell in several papers has contended that thrombokinase, or the thromboplastic substance of the tissues, is not concerned in the activation of the prothrombin, but exerts its favoring influence upon coagulation by neutralizing the antithrombin present in blood. This latter author[1] has shown, moreover, that the active constituent of thromboplastic substance is one of the lecithans or phosphatids; not the one designated as lecithin, but a related substance which in its solubilities coincides rather with the fraction known as kephalin. A somewhat similar conclusion in regard to the nature of the thromboplastic substance has been reached independently by Zak.[2]

In order to determine whether or not the thromboplastic substance (kephalin) acts as a kinase, it would be desirable to isolate prothrombin and determine directly whether calcium alone suffices to convert it to thrombin, or whether the action of thromboplastic substance is needed in addition. Unfortunately no method of isolating prothrombin has been devised, so that this direct mode of approaching the problem is not feasible at present. An indirect method of attack is suggested by the fact, emphasized by Morawitz,[3] that the favoring influence of thromboplastic extracts upon the coagulation of blood is not exhibited when the blood is deprived of its calcium

[1] Howell: This Journal, 1912, xxxi, p. 1.

[2] Zak: Archiv für experimentelle Pathologie und Pharmakologie, 1912, lxx, p. 27.

[3] Morawitz: Handbuch der Biochemie, 1909, ii, pt. 2, p. 51.

by the addition of oxalate solutions. Thromboplastic substances (tissue extracts) have a very remarkable influence in hastening the coagulation of peptone-bloods. It is upon such bloods in which, owing to an excess of antithrombin, spontaneous clotting is greatly delayed or entirely prevented, that the effect of thromboplastic extracts is shown most clearly, and it is a matter of interest to know whether in peptone-blood the presence of calcium is absolutely necessary for this action of thromboplastic substance. At Dr. Howell's request we undertook to study this point. We began our experiments with the idea that a peptonized blood which was oxalated as soon as it was drawn from the animal might behave differently from one which, after removal from the animal, was allowed to stand for a certain time, thirty minutes to an hour, before being oxalated, since in the latter there would be an opportunity for the conversion of some of the prothrombin to thrombin. To test this idea a dog was peptonized by the injection of peptone in amounts equal to 0.4 gm. per kilogram of animal. The blood, after twenty minutes, was withdrawn in two lots. The first lot was drawn at once into a solution of sodium oxalate in the proportion of nine parts of blood to one part of oxalate (one per cent sodium oxalate made up in 0.9 per cent solution of sodium chloride). The second lot was allowed to stand for thirty minutes to one hour and was then mixed with sodium oxalate in the same proportions as in the first lot. Both lots were then centrifugalized to obtain a clear plasma. It was believed that the second lot, because of standing for a time before its decalcification, would have enough thrombin formed so that kephalin solutions added to it might be able to cause clotting in the absence of calcium. The experiments carried out in accordance with this plan failed to demonstrate the point. Neither the plasma of lot one nor that of lot two would clot upon the addition of solutions of kephalin alone. Addition of calcium chloride alone caused prompt clotting, when the plasma was first diluted with an equal volume of water, and calcium solutions together with kephalin solutions were even more effective. A single example (see opposite page) will suffice to illustrate this point.

Similar results were obtained from other experiments in which the calcium was removed by the addition of sodium metaphosphate, sodium citrate, or sodium fluoride.

An interesting although inexplicable result which came out of

these experiments may be referred to briefly although it has no direct bearing upon the main problem which we were investigating. In a successful peptone plasma which does not clot spontaneously, even upon the addition of an equal volume of water, the addition of solutions of calcium chloride alone does not produce clotting while solutions of kephalin alone cause clotting in a few minutes. If this same

LOT I. PEPTONIZED DOG BLED AT ONCE INTO OXALATE SOLUTION

Peptone plasma	Water	$CaCl_2$ (1%)	Kephalin solution	
1. 10 drops	10 drops	6 drops	10 drops	Clot in 2.5 min.
2. 10 drops	10 drops	0	10 drops	No clot in 24 hr.
3. 10 drops	20 drops	6 drops	0	Clot in 11 min.
4. 10 drops	20 drops	0	0	No clot in 24 hr.

LOT II. PEPTONIZED BLOOD KEPT THIRTY MINUTES BEFORE OXALATING

Peptone plasma	Water	$CaCl_2$ (1%)	Kephalin solution		
1. 10 drops	10 drops	6 drops	10 drops	Clot in 2 min.	
2. 10 drops	10 drops	0	10 drops	No decisive clot in 24 hr.	Slight membrane formation
3. 10 drops	20 drops	6 drops	0	Clot in 8 min.	
4. 10 drops	20 drops	0	0	No clot in 24 hr.	

plasma is decalcified by the use of oxalate solutions addition of calcium chloride in quantity sufficient to overcome the excess of oxalate now causes clotting, while solutions of kephalin alone are without effect. It should be added that the promptness with which calcium causes clotting under these circumstances varied with the condition of the plasma, for example, with the amount of contained antithrombin, or with the dilution. If calcium is added to the undiluted plasma clotting may occur very slowly, whereas if the plasma is diluted once or twice with water clotting takes place promptly. As is well known, dilution weakens the effect of antithrombin. Several hypotheses may be suggested to explain this result, but as they are entirely speculative it is scarcely worth while to enumerate them. This result, however, together with other known facts regarding the action of solutions of kephalin, suggested that possibly the assumed effect of kephalin upon the antithrombin in the peptone plasmas was interfered with by the presence of an excess of the oxalate. The experi-

ments were modified, therefore, by providing for the removal of this excess of oxalate. For this purpose the oxalated peptone plasma was dialyzed in collodion tubes against large volumes of solutions of sodium chloride, 0.9 per cent, the outside solution being renewed once or twice. The results obtained from this series of experiments differed somewhat in details, for reasons which were not clear, but which depended probably upon certain variations in conditions that could not be controlled, such, for example, as the varying amounts of fibrin factors in the several bloods used, the character of the dialyzing membranes, etc. The important fact is that by this means we have been able to show that solutions of kephalin alone can cause clotting in peptone plasmas free from calcium. The following experimental results may be quoted in proof of this point.

Experiment. — Fasting dog. Eight per cent solution of Witte's peptone injected under pressure into the femoral artery in amount to give 0.4 gm. per kilogram of animal. Blood withdrawn after 20 minutes. One lot oxalated at once (9 pts. blood to 1 pt. solution of sodium oxalate made up in solution of sodium chloride 0.9 per cent) and one lot oxalated after standing 30 minutes. Each lot was centrifugalized to get a clear oxalated peptone plasma. Each lot was then dialyzed over night (15 hrs.) against a solution of sodium chloride, 0.9 per cent. The plasmas, free from oxalate and calcium, were then tested as follows:

Lot I

Peptone plasma	Water	Kephalin solution	$CaCl_2$ (1%)	
10 drops	20 drops	0	0	No clot in 24 hr.
10 drops	10 drops	10 drops	0	Clot in 45 to 50 min.
10 drops	0	20 drops	0	Clot in 1 hr.
10 drops	20 drops	0	1 drop	Clot in 15 min. — feeble

Lot II

Peptone plasma	Water	Kephalin solution	$CaCl_2$ (1%)	
10 drops	20 drops	0	0	No clot in 24 hr.
10 drops	10 drops	10 drops	0	Clot in 5 min.
10 drops	0	20 drops	0	Clot in 15 to 20 min.
10 drops	20 drops	0	1 drop	Clot in 15 min.

The kephalin solution used in these experiments was calcium free as was demonstrated by incinerating a large amount of the kephalin used and dissolving the ash in dilute hydrochloric acid. This solution gave no precipitate on the addition of ammonia and ammonium oxalate. It is not possible, therefore, to explain the clotting obtained with the solutions of kephalin on the hypothesis that the kephalin acted as a thrombokinase in conjunction with calcium as demanded by Morawitz's theory. Nor is it possible to assume that the kephalin acted as a kinase to activate alone the prothrombin present in the plasma. Other experiments, made with oxalated normal (non-peptonized) plasma, subsequently dialyzed for twenty-four hours to remove excess of oxalate, showed that the kephalin has no such action. Such plasmas clot readily on the addition of thrombin or of calcium solutions but are entirely unaffected by solutions of kephalin.

The only hypothesis that explains satisfactorily the result obtained is that the kephalin by neutralizing the antithrombin contained in the peptone plasma allowed the thrombin present to react with the fibrinogen. The specimen — lot II — allowed to stand before oxalating clotted more readily than the other specimen — lot I — because more thrombin had formed. Some subsequent experiments carried out in a manner similar to the one described above gave a different result in that the solutions of kephalin did not cause clotting in the dialyzed plasma. Investigation showed that one difficulty lay in the length of time that the oxalated plasma was submitted to dialysis. As is well known thrombin is easily adsorbed and it is, therefore, probable that in plasma in which little thrombin is present this substance may be removed partially or completely either as a result of dialyzing off or because of adsorption by the substance of the dialyzing tube. If such an action takes place kephalin of course can no longer induce coagulation, if its action is limited to neutralizing the antithrombin. In later experiments, therefore, the plasma was examined from time to time during the dialysis to determine whether oxalate was still present and whether kephalin solutions caused coagulation. While each specimen of peptoneplasma used gave somewhat different results when treated by this method, it was possible in all cases to demonstrate that for a certain time after dialysis had proceeded the plasma when treated with kephalin alone gave a clot. Before the dialysis the kephalin had been ineffective and usually

when the dialysis had gone over a certain period the kephalin was again without effect, probably, as suggested above, because the ready-formed thrombin in the plasma had been destroyed or removed. Two examples may suffice to indicate the variations exhibited by different plasmas. In each experiment the dog was peptonized as described above and the withdrawn blood after standing was oxalated and centrifugalized to obtain a clear plasma. It was shown first that this oxalated plasma was not clotted by solutions of kephalin and it was then submitted to dialysis and tested from time to time.

I

Period of dialysis	Peptone plasma	Water	Kephalin solution	
15 min.	10 drops	10 drops	10 drops	Clot in 26 min.
30 min.	10 drops	10 drops	10 drops	Clot in 41 min.
60 min.	10 drops	10 drops	10 drops	Clot in 37 min.
120 min.	10 drops	10 drops	10 drops	No clot

At each interval a control, consisting of 10 drops of plasma and 20 drops of water, was prepared. Each remained unclotted for twenty-four hours.

II

Period of dialysis	Peptone plasma	Water	Kephalin solution	
15 min.	10 drops	10 drops	10 drops	No clot
30 min.	10 drops	10 drops	10 drops	No clot
45 min.	10 drops	10 drops	10 drops	Feeble clot over night
60 min.	10 drops	10 drops	10 drops	Feeble clot over night
90 min.	10 drops	10 drops	10 drops	Firm clot over night.
120 min.	10 drops	10 drops	10 drops	No clot but precipitate

Controls with water alone (peptone plasma 10 drops, water 20 drops) tried throughout the experiment gave a negative result in each case.

These dialyzed plasmas after dilution were tested also by the addition of solutions of calcium chloride. As would be expected this reagent caused clotting in most cases within a few minutes owing to its effect in activating the prothrombin present in the plasma. Since in most cases this reaction was obtained even after prolonged dialysis

lasting over several days it is evident that prothrombin unlike the thrombin is relatively stable. In one case, however, for reasons which were not apparent, the reaction with calcium was slower and slower as the dialysis proceeded and disappeared entirely after two hours.

In the above-described experiments it is assumed that the kephalin alone causes clotting of a calcium free plasma because by antagonizing or neutralizing the antithrombin it permits whatever ready-formed thrombin may be present to exert its action On this view it is evident that if thrombin is added to the peptone plasma, in amounts insufficient to neutralize the antithrombin, the effect of the kephalin in causing clotting should be shown more clearly. Experiments demonstrated that this inference is correct. For example:

Experiment.—Dog weighing 7½ kg. Injected into the femoral artery 3 gm. of Witte's peptone. After twenty minutes the blood was drawn from the carotid into an oxalate solution (9 parts of blood to 1 part of oxalate 1 per cent). The oxalated blood was centrifugalized at 3000 for thirty minutes. The clear plasma was drawn off and was dialyzed in a collodion tube against a solution of sodium chloride 0.9 per cent until the oxalate was completely removed. With this plasma the following experiments were performed in duplicate.

Peptone plasma	Water	Solution of thrombin	Solution of kephalin	Time of clotting
10 drops	15 drops	15 drops	5 drops	4½ min.
10 drops	10 drops	15 drops	10 drops	5½ min.
10 drops	20 drops	15 drops	0	Clot between 8 and 16 hr.
10 drops	25 drops	0	10 drops	No clot in 24 hr.
10 drops	35 drops	0	0	No clot in 24 hr.

It will be seen from this experiment that while the amount of thrombin added, 15 drops, caused clotting only after eight hours, the same amount of thrombin together with 5 or 10 drops of the kephalin caused prompt clotting in four to five minutes. In this plasma, owing to the promptness with which it was oxalated and centrifugalized, none of the prothrombin apparently was converted to thrombin or if so the latter was removed in the dialysis, since kephalin alone caused no clotting.

A word may be added in regard to the action of the oxalate in retarding the normal reaction of kephalin. This retarding influence

is indicated clearly enough by the experiments described above, but other observations have shown that even in the presence of an excess of oxalate the effect of kephalin in neutralizing antithrombin may be demonstrated, provided sufficient active thrombin is present in the mixture. In one series of experiments, for example, a peptone plasma was oxalated and was then divided into two portions. To one of them was added an equal volume of water, to the other an equal volume of a solution of kephalin and to these two portions a thrombin solution was added in increasing amounts to determine for each the minimal amount of thrombin requisite to cause clotting. In all cases the solution containing the kephalin clotted with the fewer drops of thrombin indicating that in spite of the oxalate the kephalin continued to exert some favoring influence. Under the conditions of the experiment the nature of this favoring influence can hardly be interpreted otherwise than on the hypothesis that it exerted a neutralizing effect of some kind upon the antithrombin.

Summary

Calcium-free (oxalated) peptone plasma may be made to clot by the addition of calcium-free solutions of thromboplastic substance (kephalin), provided the excess of oxalate is removed by dialysis, properly controlled.

This action of the kephalin is demonstrated more easily if some thrombin is added previously to the dialyzed oxalated plasma in an amount insufficient in itself to overcome the effect of the antithrombin.

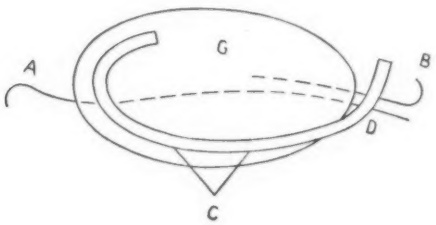
This result is opposed to the theory (Morawitz) that the thromboplastic substance acts as a kinase in conjunction with calcium, but is in accord with the view (Howell) that thromboplastic substance (kephalin) facilitates clotting by neutralizing the action of antithrombin.

ERRATA

in June number of the American Journal of Physiology (Vol. XXXII., No. II).

Substitute "*apparatus*" for "*apparati*" in the following places:

page 110, lines 7, 11, 23.
page 129, line 1.
page 137, line 28.
page 138, lines 1, 3.
page 144, line 6.

Substitute "*7.1 c.c.*" for "*1.1 c.c.*" on page 144, line 29.

In figure 1, page 120, correct as indicated the following drawing: